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FORM-REGULATION IN CERIANTHUS, IX.

Regulation, Form, and Proportion.

C. M. CHILD.

Regeneration and Proportion.

In the second paper of this series (Child, '03*b*) attention was called to the fact that the amount of regeneration is not proportional to the size of the piece. Small pieces above a certain limit regenerate as rapidly as pieces many times their size up to a late stage. Finally the small pieces fall slightly behind those of large sizes, probably as was suggested, because of the exhaustion of the material available for regeneration under the particular conditions (relative exhaustion, Child, '03*b*). Evidently the exhaustion will occur earlier, other conditions remaining the same, as the size of the piece decreases. Consequently there is some difference in the amount of regeneration according to the size of the piece, but this difference appears only at late stages and is very much less than the difference in size between the pieces. In other words the regenerated structures of small pieces are always relatively larger than those of large pieces.

The two series 54 and 55 which were described fully in my second paper (Child, '03*b*) illustrate this fact so well that it is desirable to recall certain points in this connection.

In the preparation of the two series the disc and œsophagus were removed by a transverse cut just aboral to the œsophagus and then the remaining portion of the body was cut into two pieces *A* and *B*. In Series 54*A* the oral piece was about four times as long as *B* at the time of section, while in series 55 the aboral piece *B* was about four times as long as *A*. Ten specimens were used for each series. In the pieces *A* of the two

series we have pieces ending orally at the same level of the body, but of very different lengths. For purposes of direct comparison as regards rapidity and amount of regeneration pieces must always end at the same level of the parent body for the rapidity and amount of regeneration differ markedly with the difference in level (Child, '03*b*).

Comparing 54*A* and 55*A* it was found that, although the former pieces were about four times as long as the latter, the regeneration at the oral end was the same in both during more than a month. Finally in the latest stages the shorter pieces fell slightly behind the others, but even then the difference in length of tentacles in the two sets was only 1–2 mm. Figs. 1 and 2[1] represent diagrammatic sections of 54*A* and 55*A*, thirty-one days after section, when regeneration was about completed—the experiment was performed during December and January, consequently the total amount of regeneration was much less than in the summer specimens. The length of the tentacles in the figures is the average length for the pieces of each set, but since there was little difference in individual pieces the figures would serve equally well to represent almost any of the pieces of the two sets.

FIG. 1.

The marginal tentacles in the pieces 54*A* (Fig. 1) were 7–8 mm. in length, while those of 55*A* (Fig. 2) were 6–8 mm. in length, *i. e.*, in some of the shorter pieces the tentacles were slightly shorter than in the long pieces, probably in consequence of the relative exhaustion of these pieces (see Child, '03*b*). The labial tentacles show scarcely any perceptible difference; the average length of these in 54*A* is 1.25 mm. and in 55*A* 1 mm. or a little more, but as these vary more

FIG. 2.

[1] All the figures of this paper are diagrammatic outlines drawn from measurements of the living specimens. The stippled region at the aboral end represents the aboral new tissue. It is represented as if the body were seen in surface view, not in section, in order to indicate the shading in color between new and old parts and the absence of a sharp distinction. The oral end of the body is represented in section but the œsophagus is not drawn in most cases since it was usually impossible to determine its length in regenerating specimens.

in a single piece than do the marginal tentacles the very slight difference is not important.

Aborally the amount of regeneration was much greater in the smaller pieces 54*A* than in the larger 55*A* in consequence of the difference in position or level of these ends with respect to the animal from which they were taken (Child, '03*b*). Considering the relative amount of regeneration in the two pieces we find that it is much greater in the smaller pieces. But the important point for present purposes is the absence of proportionality.

Another series, Series 46, affords similar results. In this series disc and œsophageal region were removed from twelve large specimens by a transverse cut just aboral to the œsophagus. Four of the pieces (Ser. 46*A*) were kept without further operation, four others were cut in half and the distal half used (Ser. 46*B*) and from the oral ends of the remaining four small cylindrical pieces about 5 mm. in length were cut (Ser. 46*C*). Thus three sets of pieces were obtained all with oral ends representing approximately the same level of the original specimens. The pieces *A* were about four fifths of the total length of the body, the pieces *B* about two fifths, and the pieces *C* about one fifteenth or less. During the earlier stages of regeneration there was no appreciable difference in the rate or amount of regeneration in the three sets except as regards two pieces of *C* which broke up into smaller pieces and did not regenerate at all, a common phenomenon in pieces near the lower limit of size. Five weeks later when regeneration was complete the marginal tentacles of the pieces *A* were 7–8 mm. in length, those of *B* 6 mm. and those of *C* 4–5 mm. The differences in the labial tentacles were similar. While the length of the tentacles in the smaller pieces is not as great absolutely as in the larger it is relatively much greater. The pieces *C* were only about one twelfth the length of the pieces *A* but they regenerated tentacles more than half as long. Moreover, if we compare these pieces with the entire animals in which the marginal tentacles vary in length from 25–35 mm. we find that pieces one fifteenth of the size of an entire animal and taken from a region some distance from the oral end, *i. e.*, from a region where the power of regeneration is less than at the oral end, regenerate tentacles one seventh of the maximum

length. However much the small pieces may elongate and decrease in transverse diameter the tentacles remain "too large."

Various other series of my experiments afford similar results, but these two cases are sufficient as illustrations.

Stated in general terms the conclusions drawn from the experiments are as follows: as the size of the piece decreases the relative amount of regeneration increases — provided of course that the level at which regeneration occurs is the same in all cases. It follows from this that the form, *i. e.*, the proportions, of regenerated specimens may differ widely from the typical form and that either reduction of the regenerated parts or extensive new growth in the longitudinal direction must occur. Reduction of the tentacles occurs, as has been noted (Child, '04*e*) but it is due to change in external rather than internal conditions. If the pieces are fed they will of course in time attain approximately the typical proportions. But the important point is that the regenerative processes in absence of food are not proportional.

I am inclined to believe that these facts indicate that the stimulus to regeneration is as great in small as in large pieces; that the difference in absolute amount of regeneration is due to the earlier relative exhaustion of the smaller pieces, *i. e.*, the absence of energy available in the presence of a given stimulus; and that finally if this material could be supplied in such manner as to be available only for the regeneration of a particular set of structures, *e. g.*, the tentacles, they would continue to grow until equilibrium resulted between formative stimuli and destructive factors, no matter what the size of the piece, provided it was above the minimum. In other words, it is probable that the decrease in the absolute amount of regeneration in *Cerianthus* with decrease in size of the piece is due merely to decrease in amount of available energy and not to any inherent capacity for proportional regeneration. Furthermore, it is probable that various other cases of so-called proportional regeneration are in reality similar in nature to this case.

Regulative Changes of Proportion in the Old Parts.

The next subject which requires consideration is the change of form of pieces as a whole. A cylindrical piece cut from the body is of course relatively shorter and less slender than the

typical animal. From this piece tentacles regenerate at one end and more or less growth of new tissue occurs at the other, but neither of these is proportional to the size of the piece. It has already been shown that the oral regeneration is too great in amount to permit the reduction of the piece to the typical proportions, but there remains the further question as to whether the piece shows any approximation to typical proportion, or in other words whether changes in proportion of the old parts of the piece occur which can be interpreted as constituting an approach to the typical form.

In order to answer this question careful measurements of the pieces repeated at frequent intervals are necessary, but measurement is a difficult and uncertain matter in connection with *Cerianthus*. The form varies so greatly with different degrees of distension and with the different activities of the animal that it is impossible to obtain anything more than a rough approximation. I have, however, carried out several series of measurements extending over two months or more and have succeeded, I think, in minimizing some of the sources of error. In making these measurements various precautions were observed; the pieces were absolutely undisturbed for several hours, or in most cases for nearly a day before the measurements were made, since it is only in this way that anything like complete distension and elongation can be obtained. Measurement was made by immersing a small millimeter measure in the water with the specimen, care being taken not to touch it, for a touch is likely to cause instant contraction. All measurements were repeated several times at intervals during the day, for there is no certainty that the animal is fully distended at the time of a given measurement. From these different measurements the maximum series was selected as showing the largest size attained by the specimen during a given day. The following dimensions were measured for each piece so far as the parts designated were present; the whole length of the piece from disc to aboral pore; the length of the marginal tentacles; the length of the labial tentacles; the length of the aboral outgrowth of new tissue; if such were present; the diameter of the disc, *i. e.*, the distance between bases of tentacles on opposite sides; the diameter about half way between the disc and the middle of the piece, designated as oral diameter;

the diameter at the middle in some cases where it differed from the oral diameter; and finally the diameter about half way between the aboral end and the middle, designated as aboral diameter. At the same time diagrams were drawn showing any pecularities or special features of the regeneration which might occur. All the figures of this section were drawn from these measurements and diagrams. In cases where the tentacles differed markedly in length on different parts of the circumference the two tentacles of the figure represent the extremes.

No one can be more fully aware than myself that these measurements are not and cannot be more than rough approximations. Direct measurement of the labial tentacles was often impossible, but an estimate of their length was made in such cases by placing the measure as near to them as possible and making careful comparison. It is probable, on the other hand, that these measurements are as exact as the instability of form in *Cerianthus* will permit us to obtain. While I do not believe that extended investigation along this line is profitable in this case in consequence of the absence of stable form I have proceeded far enough to convince myself that such measurements are unable in a general way. Marked differences can certainly be detected.

The results obtained from the several series of measurements made agree in general though they showed some differences in detail, and I think it is possible to draw certain conclusions from them. The following series is selected as an example from among those made.

Series 36.

On October 20, 1902, disc and tentacles were removed from a large specimen by a transverse cut, and the body was then cut into four pieces, *A*, *B*, *C*, *D* (Fig. 3). These pieces were measured at intervals of two or three days during about six weeks.

The following table is an abstract of the measurements. The proportions of the whole animal before section, the estimated lengths of the pieces cut and the dimensions of these pieces on five different dates during the six weeks are given in millimeters. These are sufficient to show the general trend of the changes and measurements made in the intervals between these dates have therefore been omitted as unnecessary for the present purpose.

Date.	Piece.	Length of Body.	Length of Marginal Tentacles.	Length of Labial Tentacles.	Length of Aboral New Tissue.	Diameter of Disc.	Oral Diameter of Body.	Middle Diameter of Body.	Aboral Diameter of Body.	Figure.
Oct. 20, '02. Before Section.	Whole.	95	30	12–15		12	7	6	5	Fig. 3
Oct. 20, '02. At time of Section.	*A*	25								Fig. 3
	B	28								" 3
	C	12								" 3
	D	25								" 3
Oct. 21, '02.	*A*	9					5		6	Fig. 4
	B	15					6		6	" 9
	C	5					5		5	" 14
	D	10					5		3	" 19
Oct. 29, '02.	*A*	15	1–1.5				6	7	6	Fig. 5
	B	20					5	6	6	" 10
	C	6					4		4	" 15
	D	22					4	6	4	" 20
Nov. 6, '02.	*A*	10–12	7–8	0.5–1	1	6.5–7	6		6	Fig. 6
	B	18	4–5	0.5		6	5		5	" 11
	C	8–9	0.5–2			5	5		4.5	" 16
	D	18	0.5			4.5	4.5		4	" 21
Nov. 20, '02.	*A*	18	8–10	2–3	3	6	5		3.5	Fig. 7
	B	27	10–12	3–4	3	6.5	4		4	" 12
	C	10	5–8	1	2	5	4		4	" 17
	D	25	8–10	2		5	4		3	" 22
Dec. 2, '02.	*A*	27	12	3–4	3–4	6–7	4.5		3.5	Fig. 8
	B	28–30	12–14	3	3	6	4.5		4	" 13
	C	18	8–9	2–3	2	5	3		2.5	" 18
	D	25	11–12	3		5	3.5		3	" 23

A horizontal reading of the table gives the measurements for each piece at each date and the last vertical column headed "figure" gives the number of the text figure representing the piece at the stage indicated by the horizontal division.

It is readily seen from the table and figures that the length of each piece gradually increases while its transverse diameter gradually decreases during the course of the experiment. The length of the tentacles increases during the whole period. There are several irregularities in the table which are undoubtedly due to the great changes in form in individual specimens: for example, piece *A* is shorter on November 6 than on October 29. This difference is merely temporary and due to the fact that the piece is not as fully extended at the second measurement as at the first. But the general result is sufficiently clear from both table and figures. The extremely small size of the specimens on the day

after section is of course due to complete collapse. The pieces in this condition are not strictly comparable to the distended pieces.

The data indicate that a marked change in form occurs in these pieces and that this change consists in an approach to the typical proportions; although none of the pieces attain them. For example the piece *B* (Fig. 13) approaches most closely at

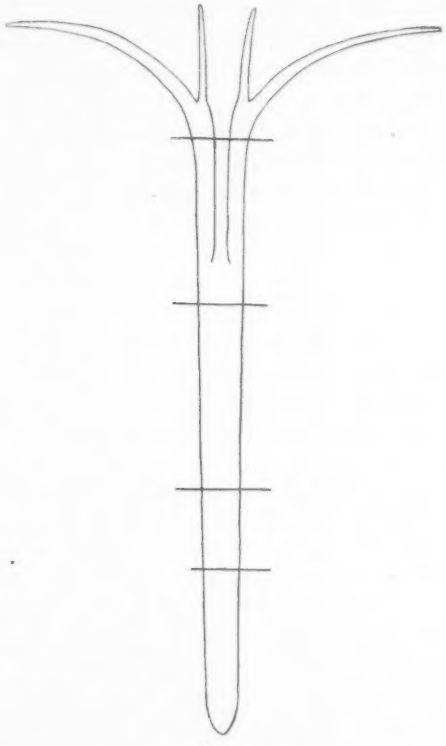
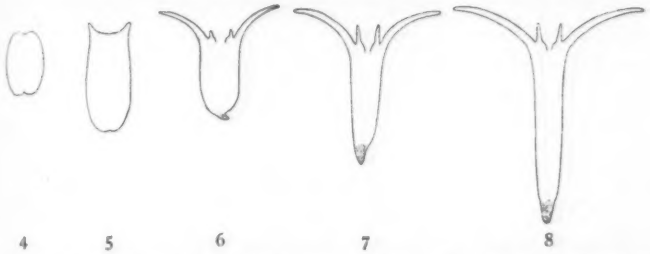


FIG. 3.

the end of the experiment to the original proportions of the whole before section, but even here the tentacles are relatively longer, the body-length is relatively less or the transverse diameter is relatively greater than in the whole. In the short piece *C* (Fig. 18) the proportion between length and transverse diameter is 6:1

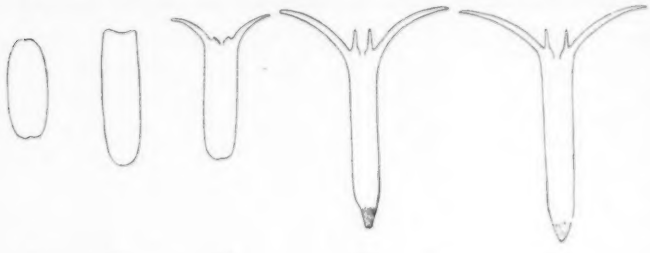
as compared with about 13.5:1 in the whole. The relation between length of marginal tentacles and length of body is about 1:2 while in the whole it is 1:3. The change in the relation between length and transverse diameter in each piece during the experiment consists chiefly in decrease in the latter. None of the pieces except *C* have increased in length to an amount equal to the growth of new tissue at the aboral end. In other words all but *C* both length and transverse diameter of the old tissue have decreased but the latter much more rapidly than the former. In the actual decrease in size two factors are probably concerned, viz., loss of substance and decrease in general internal pressure: of these the former is due to starvation and use of material in



regeneration, the latter both to approaching exhaustion and to the reduction in temperature which occurred during the course of the experiment from October to December; both of these conditions reduce ciliary activity and therefore internal pressure also. But these factors alone must bring about a proportionate or nearly proportionate reduction in form. Other factors must therefore be concerned in the change of form which occurs in these pieces.

It remains then to determine whether or not this change of form is due to some inherent capacity of the protoplasm independent of external conditions. The first point of importance in the consideration of this question is the dependence of the change of form upon distention with water. Pieces kept open at one or both ends continue indefinitely to grow shorter and smaller until finally they form rounded masses, even though originally the length was many times the diameter; in short the changes in these collapsed pieces are opposite in character to those that occur in

the pieces described above. The reduction of collapsed pieces has been mentioned several times in this series of papers (see especially '04*b*) and need not be discussed in detail again. The fact that the relation between length and transverse diameter changes in opposite directions in collapsed and distended pieces is important and may, I think, be taken to indicate that the distention with water plays some part, either directly or indirectly, in the "elongation" which distended pieces undergo. Two possibilities suggest themselves to me in this connection, viz.: either the pressure of the water in the enteron constitutes in some way a stimulus to elongation or the change of form is an

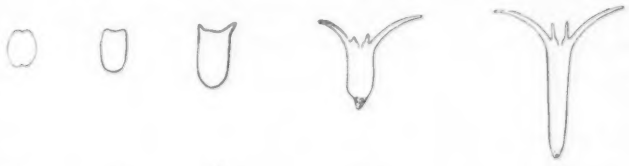


9 10 11 12 13

incidental result of the behavior which is markedly different in distended and collapsed pieces.

Regarding the first of these possibilities, viz., internal pressure as a stimulus to elongation I can offer no experimental evidence. I desire merely to call attention to certain points. It is evident that the general internal pressure cannot constitute a stimulus to elongation unless we postulate a difference in the reactive capacity of the tissues in different directions; it may be that such a difference exists but I know of no basis of fact for this assumption. On the other hand it is possible that currents in the enteron might exert local pressure in particular directions. It has already been noted in numerous cases (Child, '04*b*, '04*c*, '04*d*, '04*e*, '05) that there is some ground for the belief that circulatory currents (Child, '04*b*) may constitute factors in the localization of regeneration, *i. e.*, that the local pressure may act as a stimulus to growth. Currents passing orally along the inner surface of the body wall in all the intermesenterial chambers must produce a

total pressure of considerable amount upon the oral end. On the other hand a current passing aborally along the inner margins of the mesenteries or a part of them must finally strike the aboral end and produce pressure there. Now the effect of these currents may conceivably show itself partly in local growth in region of impact, *i. e.*, where the tension upon the tissues resulting from the impact of the current is greatest and partly in a diffuse growth or a change of form in accordance with the tension in the surrounding regions to which some degree of the tension is transmitted. Since these currents strike the terminal regions of the body local growth at the ends and elongation of the whole must be the result if they are effective in the manner suggested. In pieces kept without food the elongation must be accompained



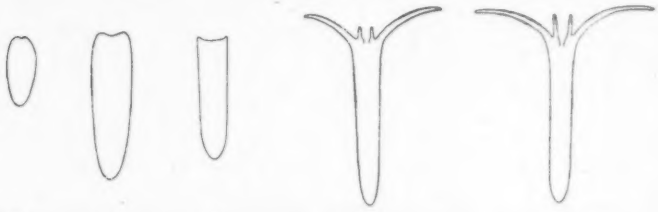
14 15 16 17 18

by a reduction in the transverse diameter. These suggestions as to the effects of circulatory currents, like those made in previous papers (Child, '04*b*, *et seq.*) must be regarded at present as merely tentative. They may be entirely incorrect but I think the hypothesis of the effectiveness of circulatory currents as localizing formative stimuli cannot be rejected off-hand. It serves so well as a basis for explanation of various phenomena that it must at least receive consideration. I hope at some future time to obtain experimental evidence regarding this hypothesis.

The second possibility, viz., that the change of form may be an incidental result of the movements of the animal must also be considered. As noted above, the behavior of typically distended specimens is very different from that of the same pieces during collapse. The creeping habit of *Cerianthus* is well known; the animals are able to move about freely, lying upon the side and moving with either oral or aboral end in advance. They move not only on horizontal surfaces, but are able to climb the vertical sides of a glass jar or aquarium. In my aquaria specimens often

climbed two feet or more up the vertical side. Cilia afford of course the motive power and the slime secreted serves as a means of adhesion to surfaces. Observation of creeping pieces shows clearly that the body is subjected to more or less tension in the longitudinal direction during this movement. Probably the slime secreted by the ectoderm is responsible for most of this tension. I think it possible that this tension may bring about some degree of elongation in much the same way as in *Stenostoma* (Child, '02, '03).

In *Cerianthus* the tension is due rather to the slime secreted than to the use of the end as an organ of attachment, as is the case in *Stenostoma*, but the result, viz., longitudinal tension upon the tissues, is similar in both cases. If the tissues here are af-



19 20 21 22 23

fected by the tension as they are in the turbellaria, elongation must result, whether from growth or from mechanical rearrangement of the tissues or cell-elements.

Soon after beginning my experiments I found that collapsed specimens and pieces in early stages of regeneration crept about very little. It was found that such pieces could be kept without danger of loss in Stender dishes with sides 7–8 cm. in height, which were placed on the bottom of large tanks filled with water; the pieces never crept out. Later, however, the same pieces would creep out of battery jars with sides 30–40 cm. or more in height. In these respects I noted a difference between those pieces which still retained the original oral end and tentacles and those in which oral structures were in process of regeneration. Pieces of the first sort began to creep about soon after closure of the aboral end and distension occurred, *i. e.*, within a few days in many cases, while the pieces with regenerating oral ends usually

moved very little during the first three to four weeks after section, *i. e.*, before regeneration of the oral structures was well advanced. In short, the collapsed specimen and the specimen — collapsed or not — in which the oral structures are not well developed, do not exhibit the typical behavior of the species as regards movement. Probably in the one case the absence of distension brings about some change in the internal stimuli which inhibits the motor impulses to a greater or less extent. In the other case the most highly organized portion of the body — the oral region — is absent and the reactions of the pieces are much less vigorous and effective.

To sum up: two possible factors in the elongation of pieces have been suggested; the one, tension upon the terminal regions of the body in consequence of the impact of enteric circulatory currents against the wall; the other, tension upon the tissues in the longitudinal direction in consequence of movement over surfaces accompanied by some degree of adhesion.

It is impossible to determine at present whether one or the other of these factors is chiefly concerned, or whether both play important parts in the change of form. It may be that other unknown factors are concerned, but it is highly improbable that there is any mysterious internal ultra-physico-chemical factor which brings about a characteristic form. Mention has already been made of the fact that collapsed pieces never undergo this change of form, but depart farther and farther from the "typical" form. Now it is possible to keep a specimen collapsed by means of a very small opening at one end or elsewhere, provided this be reopened frequently. It is difficult to understand how such an opening should interfere with any internal or "vitalistic" factor capable of causing return to the typical form. Here again experiment indicates the impossibility of explaining the facts with the aid of Driesch's hypothesis (Driesch, '01, '03).

Both of the hypotheses suggested belong in one category, viz., that of mechanical tension. Whether this tension brings about simply mechanical deformation of the tissue elements, or whether it acts as a functional stimulus to which the tissues react by growth, cannot readily be determined. Probably both mechanical deformation and growth occur; indeed, it is probable that the former brings about the latter.

According to this view the elongation of pieces of *Cerianthus* and the extremely elengated form of the body of normal animals are due to the same factors.

The tubicolous habit of the animal may perhaps be regarded by some as responsible in some degree for its elongated form. In the tube only longitudinal increase in size can occur. The animal frequently leaves its tube, however, and burrows in another spot; the size of the new tube will be determined by the size of the animal in each case. Moreover, I do not think the reduction in transverse diameter observed in pieces can be explained in this manner. The slime which encloses the bodies of specimens kept in clear water is not sufficiently elastic to reduce the diameter. In so far as the burrowing tubicolous habit is correlated with the creeping habit, it may constitute a factor in the production of the characteristic elongated form.

One point still requires brief consideration. The table and the figures indicate that piece *C* undergoes a much greater change of form than the other pieces. It is the only piece in which the length of the old part is greater at the end of the experiment than at the beginning, *i. e.*, provided the measurements are correct. There is of course uncertainty regarding all the measurements but if we suppose this difference between *C* and the other pieces to be real it requires explanation.

In my study of *Stenostoma* (Child, '02, '03) it was shown that the change in direction of the tension upon the tissues is an important factor in change of form. In a short piece of *Stenostoma* using its posterior end as an organ of attachment the directions of the various strains to which the tissues are subjected differs much more from those to which they were subjected as a part of the whole than would be the case in a long piece. This must be true in all cases except where the posterior end of the piece formed the posterior end of the whole. In that case the directions of the strains remain as before. For further consideration of the case the reader is referred to the above-mentioned paper. The point of importance for our present purpose is that the short piece must undergo greater change of form than the large piece except when it is taken from the posterior end.

We can I think, apply the same course of reasoning to *Ceri-*

anthus. If we consider animals moving about, oral end in advance as they very commonly do, a cylindrical portion of the body situated at any region except the extreme end, is, let us assume, subjected to a certain degree of tension in consequence of the movement. Now since this piece is attached to other parts both at its oral and aboral ends about its whole circumference the strains to which its tissues are subjected are almost wholly parallel to the longitudinal axis. If now the piece be removed from the whole and allowed to regenerate in the typical manner closure of the ends and outgrowth of new tissue at the aboral end occur before the piece begins to move about. If now the piece begins to creep and is in consequence subjected to tension the elements of this tension affecting various parts will differ not only in amount but also in direction because the piece is no longer attached to other parts at both ends by its whole circumference. Comparing a long and a short piece it is clear that the change in direction of the strains in the long piece considered as a whole is very much less than in the short piece. We may expect, therefore a greater change of form in the short piece than in the long piece, after equilibrium is attained. The greater change in the piece *C* as compared with *A*, *B*, and *D*, which I am inclined from comparison with other pieces to regard as real, may then be explained on the same basis as similar changes in other creeping forms. The fact, shown by the figures and table, viz., that the change of form became much more rapid toward the end of the experiment, *i. e.*, in the fifth and sixth weeks, agrees well with the fact that the piece did not begin to move about freely during the first month after section. During this time it never climbed out of the jar, but during the next two weeks it climbed out of a deep jar into the large tank almost daily and wandered about the tank. As often as found outside it was returned to the jar. It was during this time that the chief change of form occurred (compare Figs. 17 and 18). These facts may perhaps indicate that the movements are more important than the internal pressure in bringing about the change of form.

In *Cerianthus* then we find another case in which the assumption of a mysterious formative principle is in no way necessary. Form in the lower animals is probably to be considered as rela-

tively simple. It should be borne in mind, however, that cells and tissues of a certain constitution, *i. e.*, *Cerianthus*-protoplasm, must be acted upon in order that a certain result characteristic of the species may be obtained. In another species the result of a stimulus similar in degree and kind may be more or less widely different. At best the problem of form is complex but I believe that in general we must look to function for an explanation of form, whether direct or indirect, and not to form for an explanation of function. This opinion does not involve the paradox of differentiation of function in a structurally isotropic protoplasm, but requires a distinction between chemical and physical structure of protoplasm and morphological structure in general. Function in a general sense is an expression and result of the first, while the second is, at least in many cases, an expression and result of function.

Experimental Duplication of Parts.

By partial longitudinal splitting of the body, the separated portions remaining attached to the undivided part, it was possible to produce specimens with two oral or aboral ends as the case might be. In cases where one of the parts separated by the longitudinal cut was considerably smaller than the other, and especially in cases where the cut was somewhat oblique, the smaller part or that attached to the undivided region by the narrower portion very commonly separated from the remainder of the body within a few days and regenerated independently. In many cases also closure was much delayed by the irregular inrolling of the edges and one or both of the split portions became greatly reduced or constricted off. In a certain number of the pieces, however, the split portions roll longitudinally and their edges unite thus giving rise to the duplication. In case of duplication of the oral end the two parts are in reality "halves," each possessing approximately half the full number of tentacles. Where closure is perfect or nearly so each disc of course possesses a mouth and œsophagus formed by union of the longitudinal cut surfaces of the half œsophagus—in reality half structure. Whether in cases where one of these half structures contains the old siphonoglyphe the other ever forms a new one I do not know. I have never found a new one, but it may appear in time.

In cases of aboral duplication each part is really a "half," but the aboral pore is duplicated, each pore being formed from a part of the old pore in case the cut passed through it, or one pore being formed anew in case the cut passed more or less to one side of the old pore.

The varieties in method of closure in these cases are almost infinite as may be conceived from the discussion of inrolling in a previous paper (Child, '04*a*). Scarcely any two of the resulting forms are alike but the many differences in detail do not add essentially to our knowledge, being merely illustrations of one general principle. They do show very clearly, however, how little power the animal possesses to maintain or return to a particular form after section. All is pure chance in the matter of closure. Whatever portions of the cut surfaces come into contact unite. The tensions due to internal water-pressure may serve in some cases to modify the peculiar forms, but in other cases this does not occur. All depends upon the conditions of the particular case.

In cases where closure and duplication of the oral or aboral ends is accomplished I have never seen reduction, absorption, or loss by constriction of one of the two parts afterward. Occasionally if the duplication involves merely the extreme end it gradually disappears; the two halves gradually becoming a whole. In other cases where the duplication extends for some distance from the end so that the two parts are distinct, I have not noted any changes which might be interpreted as a regulation of the atypical form, although specimens of this kind have been kept for more than three months.

There is, no doubt, that many other varieties of monstrous forms may be produced. The only obstacle is the frequent separation of parts from the body after severe operation.

Summary.

1. Regeneration in *Cerianthus* is not proportional to the size of the piece. The smaller the piece, other conditions remaining the same, the greater the relative amount of regeneration. As regards absolute amount of regeneration the small and large pieces are at first alike, but later the small piece falls behind, *i. e.*, regeneration is retarded and ceases sooner than in the large piece, probably owing to lack of available energy.

2. Cylindrical pieces usually undergo a greater or less change of form during or after regeneration: this consists of an increase in length and a decrease in transverse diameter.

3. The change is slight during the earlier stages of regeneration before the oral structures are developed. It seems probable that it is the result, either directly mechanical or reactive or both of longitudinal tension upon the tissues. The tension in turn may be due in part to internal circulatory currents and in part to the habit of creeping over surfaces in the direction of the longitudinal axis.

4. In pieces maintained in the collapsed condition the change of form is the reverse, *i. e.*, the length decreases and the transverse diameter increases, at least relatively.

5. It is possible to produce forms with duplicated oral or aboral ends by partial longitudinal splitting: In cases of oral duplication each disc is essentially a "half" structure or fractional structure, bearing approximately the number of tentacles corresponding to the portion of the circumference which it represents. At the aboral end a new aboral pore may be formed in case the cut does not pass through the old pore.

6. No marked regulation, reduction or absorption of these duplicated structures has been observed except occasionally in cases where the duplication included only the extreme terminal portions.

7. The results of attempts to produce duplications and abnormal forms depend largely upon chance. Whatever portions of the cut surfaces come into contact unite and many peculiar forms result which may be more or less modified in some cases by the tensions and pressures to which the tissues are subjected.

HULL ZOÖLOGICAL LABORATORY, UNIVERSITY OF CHICAGO,
December, 1903.

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NOTES ON THE LIFE HISTORY OF THE STYLOPIDÆ.

CHARLES THOMAS BRUES.

During the past summer I had the opportunity of making a number of incidental observations on the two Texan species of *Xenos*, and of discovering the female of one of them, which has not hitherto been described. As these notes throw some light on the life history of these interesting creatures, I venture to present them at this time.

On May 22, at Paris, Tex., I captured a large overwintered female of *Polistes rubiginosus* which had evidently just left its hibernation quarters. Examination showed that it harbored a single female specimen of *Xenos nigrescens* Brues. The head of the parasite protruded between two of the apical abdominal segments and a number of triungulins were emerging from the small rectangular orifice on the anterior portion of the exposed surface of the head. The triungulins do not move very rapidly and cling rather tenaciously to the body of the wasp, even when the latter is shaken violently about. They keep continually in motion, however, and when the wasp is resting a number of them are always crawling off upon adjacent objects. They are rather dark brown in color and scarcely visible to the naked eye. The following description is drawn up from a number of mounted specimens:

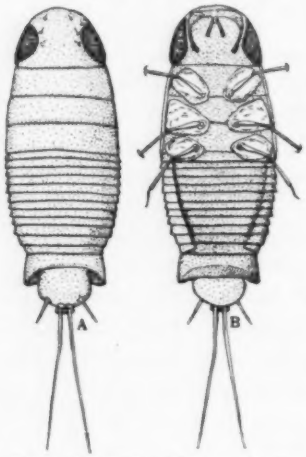

FIG. 1. Triungulins of *Xenos nigrescens* Brues. *a*, dorsal view; *b*, ventral view.

Length .28 mm. Oval, head obtusely rounded anteriorly. Head and thorax together slightly longer than the abdomen. Head a little less than half as long as the thorax, almost semicircular when seen from above, being

truncate behind. Eyes large, strongly pigmented. Oral opening large, almost contiguous with the eyes below; mouth parts consisting apparently of a short proboscis-like organ with chitinous sides. No antennæ or other tactile organs to be seen. Thorax one and one-half times as long as wide, consisting of three nearly equal transverse segments. Each segment below bears a pair of very small and delicate legs. The coxæ are all greatly swollen and globose, those of each side contiguous with one another and the pairs only moderately separated along the median line. Each coxa is hollowed out below and the trochanter sunk within it. Femora slender, enlarged somewhat at the base, bearing a spine apically. Tibiæ slender, of equal width, the hind ones with a preapical spine. Tarsi greatly reduced, those of the four anterior legs scarcely distinguishable from the tips of the tibiæ, furnished with a pulvilliform appendage; the posterior pair elongated, with a styliform appendage. Abdomen consisting of nine short, transverse segments and an elongated tenth segment which encloses the extrusible tip of the abdomen (11th segment?). The dorsal sclerites reach far down on the sides, as do also those of the thorax, making the underside of the body somewhat concave. Tip bearing two approximated bristles, each as long as the abdomen, in addition to a much shorter one at each lateral angle of the last segment.

The triungulins are strongly campodeiform in type, but show several remarkable adaptations in the shape of the legs. Aside from these peculiarities they are very similar to Meloid triungulins.

There are several differences to be noted between them and the triungulins of *Stylops* as figured by Packard ('98, 695) and Sharp ('01, 300). Principal of these is the presence of a pair of large, strongly pigmented eyes, and the structure of the legs in which the tarsi are so much reduced. In Packard's figure the different form of the anterior and posterior tarsi are shown somewhat as I have observed them in *Xenos*.

Of the three species of *Polistes* which are especially abundant in Texas, only two seem to be at all generally attacked by *Xenos*. These are *Polistes annularis* and *P. rubiginosus*. From the former both *Xenos pallidus* and *X. nigrescens* were bred in considerable numbers, and from the latter only *X. nigrescens*, and that in smaller numbers. The fewer *Xenos* obtained from the specimens of *rubiginosus* is due both to the smaller percentage of individuals infested and usually to the occurrence of only one parasite in each wasp. In *annularis* there were on an average from three to four *Xenos* in each parasitized wasp, and in one case ten fully

developed male pupæ. Although hundreds of specimens of *Polistes texanus* were examined they showed no parasites at any time during the season.

The following tabular arrangement has been prepared to show some of the more important relations betwen the *Polistes* and the parasites in two nests of *annularis*.

First nest of *Polistes annularis*; 86 female wasps, 44 of them parasitized by *Xenos nigrescens*. An examination of 36 of these revealed parasites as follows:

Wasp No.	Male *Xenos*.	Female *Xenos*.	Wasp No.	Male *Xenos*.	Female *Xenos*.	Wasp No.	Male *Xenos*.	Female *Xenos*.	Wasp No.	Male *Xenos*.	Female *Xenos*.
1	1	1	10	3	0	19	3	0	28	1	0
2	0	1	11	1	0	20	2	0	29	1	0
3	2	1	12	3	0	21	7	0	30	4	0
4	1	0	13	6	0	22	6	0	31	4	0
5	1	0	14	3	0	23	2	0	32	1	0
6	1	0	15	5	0	24	1	0	33	4	0
7	1	0	16	4	0	25	3	0	34	2	0
8	4	0	17	2	0	26	1	0	35	1	0
9	4	0	18	1	0	27	3	0	36	3	0

In the total of 36 wasps examined there were 91 male and only 3 female parasites. In two cases the females were associated with males in the same wasp. This great preponderance of males in the nest is very remarkable and may possibly be due to the season of the year (July) since the next nest to be described contained many more females and was collected much later (October 3).

Second nest of *Polistes annularis*; 42 female wasps, 36 of them parasitized by *Xeno pallidus*.

Wasp No.	Male *Xenos*.	Female *Xenos*.	Wasp No.	Male *Xenos*.	Female *Xenos*.	Wasp No.	Male *Xenos*.	Female *Xenos*.	Wasp No.	Male *Xenos*.	Female *Xenos*.
1	6^{e1}	1	10	0	2	19	0	1	28	0	2
2	6^{2e1}	1	11	0	2	20	2	larvæ	29	4	0
3	5	1	12	1^{e1}	0	21	0	1	30	4^{e1}	1
4	3	3	13	1 2	larvæ	22	1	2	31	0	1
5	10^{e1}	0	14	0	1	23	2	3	32	1^{e1}	2
6	4	2	15	2	1	24	0	2	33	6	2
7	1	2	16	1	2	25	2	1	34	0	1
8	2	0	17	1 3	larvæ	26	1	4	35	0	2
9	7	0	18	1	1	27	5^{e1}	0	36	4	1

Total males, 81. Total females, 44. Wasps with females only, 10. Wasps with males only, 8. Wasps with both males and females, 17. Wasps with larvæ only, 1.

[1] *e* refers to the number of males which had emerged when the wasp was captured.

A study of the conditions in this nest bring to light a number of interesting points. It is seen that seventeen, or almost fifty per cent. of the wasps contained both male and female parasites, while about an equal number contained parasites of only one sex. In these latter the number infested by males and females was about equal. A single wasp contained only larvæ, while several bore larvæ in addition to imagines. The presence of larvæ in full-grown wasps is no doubt due to a failure to keep pace in development with the growth of the wasps.

From the large number bearing parasites of both sexes, in seems probable that at times the sex of the parasites is in no way influenced by the host, or rather, that the sex of the specimens to mature in a single wasp from the large number of *Xenos* larvæ usually present is not always the same as I had previously supposed from former observations ('03, 246).

A second point of interest is the considerable number of wasps, seven in all, from which males had emerged before capture. From previous observations upon wasps in captivity ('03) I was led to believe that the hosts died very soon after the emergence of male *Xenos* from their bodies.[1] Such did not appear to be the case here, for one wasp which was actively feeding upon the nectary of a cotton square late in the afternoon (the males almost always leave the pupa case early in the morning), proved on later examination to bear ten empty male pupa cases between the segments of the abdomen. In some of the wasps included in the table it is quite possible that a number of the males may have emerged at time of capture or during the several hours confinement of the wasps in a screen box before careful examination. It has been remarked by Packard that the males are apt to emerge during any excitement or great muscular exertion of the host, for example when the wasp is caught in a net. This may to some

[1] In this connection it is interesting to note that this fact was commented upon in 1793 by Rossi in what is no doubt the first mention of *Xenos* in scientific literature. His statement given in the *Bulletin de la Société Philomathique*, Vol. 1, p. 49, is as follows: "Cet insecte habite à l'état de larve et de chrysalide dans la guêpe française *vespa gallica* C'est sous la quatrième anneau de l'abdomen de cette guêpe que se trouve sa chrysalide; sa présence ne nuit pas à la vie de la guêpe, et on rencontre souvent sous les anneaux de leur abdomen les chrysalides dont l'insecte est sorti, sans que les guêpes paroissent incommodées."

extent account for the discrepancy between the two series of observations.

The presence of numbers of females in the wasps during October when *Polistes* are searching for hibernating quarters suggests that the parasites pass the winter in this state. With these species as is known to be the case with *Xenos peckii*, a cursory examination under the microscope showed that the masses of eggs in several females were in a very early stage of development. From this there seems no reason to doubt that the gravid females hibernate in this state, and that the later embryonic development is passed during the early part of the following spring. It would appear very doubtful that any males hibernate. The fact that many had emerged and that those still remaining in the pupal envelopes had already acquired their adult color by October first shows almost conclusively that all would emerge before winter. Spring observations also support this idea for no males were seen in over-wintered wasps. Neither were any wasps containing empty male pupal cases seen in the spring. During the winter a large proportion of the *Polistes* die, and no doubt the ones from which males had emerged would succumb first. In the nest in question, then, only ten of the parasitized wasps could be expected to survive the winter. That the majority of these die also is shown by the scarcity of parasitized wasps in the spring.

From these facts it is evident that no males survive the winter and probable also that no wasps which have contained males survive. This necessitates the death of a large proportion of the female *Xenos* since it has been noted (*ante*, p. 292) that of 44 females only 14 were in wasps that did not contain males.

On account of the difference of opinion as to the way in which the wasps treat the emerging male parasites, a close watch was made on several occasions, but at no time could it be observed that the wasps took any notice at all of the fluttering male *Xenos*.

Quite recently Pierce ('04) has added to our list a new species of Stylopidæ described by him as *Xenos pulvinipes*. An examination of his figures and description convince me that this form is generically distinct from *Xenos* for several important reasons. According to the description the female possesses distinct eyes, a character at once distinguishing it from *Xenos*

and so far as I am aware from all other Stylopid females. It has also segmental spiracles which are absent in the other forms, and the males have very peculiarly modified tarsi.

Following is the description of the female of *Xenos pallidus*:

XENOS PALLIDUS Brues ('03).

Female. Length 8–12 mm. Distinctly more slender than *X. peckii* or *X. nigrescens*. Head black, except on the anterior third, the lines between the colors straight, transverse. Head in outline more elongate than in the other two species and less bowed on the sides, widest quite near the posterior angles (see figure 2). Thoracic integument very dark, wrinkled as usual, its posterior margin gradually slanting backwards on each side toward the median line. Dorsal stripe very distinct, always extending over seven segments as shown by its constrictions, distinctly marked posteriorly and not fading out indefinitely. Openings to the oviducts distinct, four in number, one on each of the four anterior abdominal segments near the posterior margin. Abdomen more or less distinctly constricted just before its tip.

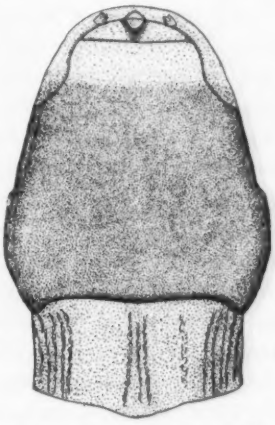

FIG. 2. Exposed portion of adult female of *Xenos pallidus* Brues.

Described from over 90 specimens found associated with the males of the species in the bodies of *Polistes annularis*.

In conclusion, I wish to thank Mr. F. C. Bishopp who collected a considerable portion of the stylopized *Polistes* considered in the foregoing notes.

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IMMATURE SPECIMENS OF PENELLA FILOSA.

M. T. THOMPSON.

The young stages of all parasitic Crustacea are of interest, and this is especially true with respect to the highly modified forms. Hence I was pleased to find among a number of *Penella filosa* loaned me by Dr. J. S. Kingsley, a few immature specimens. *Penella filosa* is the commonest species of *Penella* on our coast and in all probability is unhesitatingly to be identified with the form described from the sword-fish by Linnaeus in 1758 as *Pennatula filosa* under the impression that it was a Sea Pen, an error first corrected by Pallas eight years later. This species infests the sun-fish — *Orthagoriscus* — as well as the sword-fish — *Xiphias* — and the double host has suggested to some investigators that the name "filosa" covered an assemblage of ill-described Penellas rather than characterizing a distinct species. This view, however, does not seem well taken. With respect to the forms usually called by the name "filosa," material from either the sun-fish or the sword-fish presents no constant pecularities by which the source may be determined. My immature specimens were taken from sword-fish.

The adult *Penella filosa* frequently attains a length of 130 mm. and may be over 20 cm. long. Of this, almost one half, the round "head" and slender cylindrical "præ-thorax," is buried in the tissues of the host. Only the thicker "post-thorax" and brush-like abdomen trail free in the sea-water, furnishing a locus for a dense growth of hydroids and occasionally barnacles, *Conchoderma*. The abdomen is less than one third of the exposed part of the animal and owes its brush-like appearance to from twenty to thirty pairs of lateral appendages each of which is subdivided into numerous thread-like branches. The trailing egg-sacs are filiform and not infrequently measure 8 or 10 cm. in length. The "head" retains minute, elongate, first, and stouter, chelate, second antennæ on the dorsal surface. Mouth parts are absent and the ventral and anterior surfaces of the "head"

are tuberculated. Behind the "head" the præthorax bears four pairs of minute limbs and forward from the anterior pair of these a stout, blunt "horn" projects on either side. This arrangement of horns, however, is not invariable and out of thirty specimens at my disposal from *Orthagoriscus mola* I found nine with an additional dorsal horn. A similar variation in the number of the horns was noted in *Penella varians* by its describers (Steenstrup & Lütken, '61) and parallel conditions are frequently met among parasitic Copepoda which attach to their hosts by analogous structures. A typical specimen of the Lernæenicus of the Menhaden for example — *L. radiatus* — has five simple horns; but specimens are commonly taken which possess a greater or lesser number of these *haftorgane* and not infrequently they are forked or are rough with small branches.

The immature *Penella* in the collection loaned me were five in number. To these a young *Penella filosa* taken from a sword-fish brought into Woods Hole has been added, so that my series included six specimens. The youngest individual (*A*) (Fig. 1), had a distinct cylindrical cephalothorax, highly convex, with a double-lensed median eye near the anterior end. Beyond the border of the cephalothorax the three-jointed (?), hamate second antennæ projected. This cephalothorax was flecked with considerable black pigment. The filiform thorax extended to a length thirty-two times that of the cephalothorax. It was not segmented but ringed as in the adult with indistinct circular constrictions near the hinder end. The abdomen was filiform, bore along its sides twenty-two pairs of short unbranched lateral appendages and terminated with a deep notch. On either side of this was a small bisetose appendage. The first pair of antennæ were delicate, two-jointed, and setose. A prominent, urn-shaped rostrum with a reflected, finely serrate border projected from the venter of the cephalothorax and was flanked by reduced maxillæ in the form of minute palps each tipped with two delicate bristles. No traces of mandibles were found. Just behind the rostrum in the mid-line there was a prominent rounded tubercule and on either side of this the two-jointed maxillipeds were situated. Further back, two shallow depressions and back of these two larger shallow pits were visible.

The thoracic limbs appeared to be much like those of an adult *P. filosa* as far as it was possible to examine them. No traces of appendages other than these just mentioned were found. This

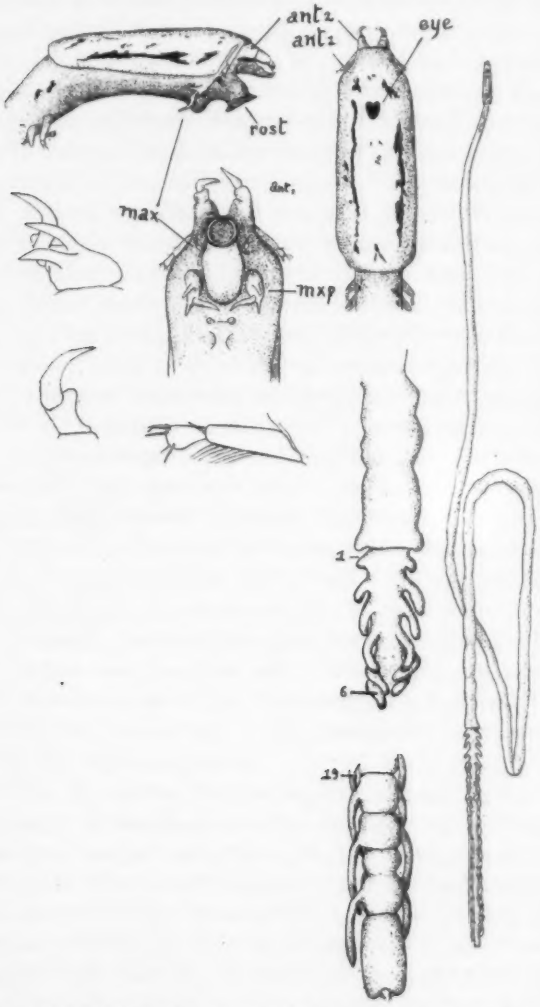

FIG. 1. Youngest larva of *P. filosa*; with three aspects of the carapace, anterior and posterior extremities of the abdomen, maxilliped, first and second antennæ more enlarged.

specimen was 94 mm. long; of which the head measured 2.5 mm., the thorax 80 mm., and the abdomen 11.5 mm.

The next older specimen (*B*) shows a marked advance in development over "*A*" (Fig. 2). The cephalothorax and thorax are no longer distinctly demarked from one another and the sides of the anterior portion of the former region are inflated so that a "head" is differentiated. On the dorsal surface, in the neighborhood of the antennæ, a trace of the borders of the cephalo-thorax still persist. In the region of the anterior pair of thoracic limbs, behind the newly-formed "head" a pair of blunt horns have made their appearance. The median eye was not noted in this individual. The antennæ and maxillipeds are much the same as in stage "*A*," except that the second antennæ are now more chelate. The rostrum is distinctly shifted toward the anterior border of the head and the median tubercule is now reinforced by a conical tubercule on either side of the rostrum. Whether this pair of tubercules replace the maxillæ or not is uncertain. These appendages were not observed in the specimen under consideration, but the minute palps to which they are reduced were doubtfully traced in the next older specimen mesad from the lateral tubercules. The abdominal appendages are uniramous as before. The length of "*B*" was 130.5 mm. The "head" measured 2.5 mm. long by 1.2 mm. wide, the thorax was 120 mm. long, the abdomen 8 mm. long.

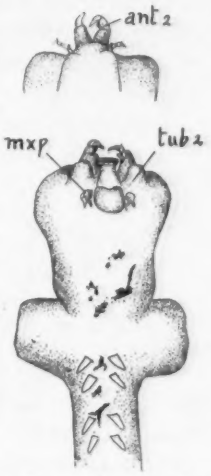

FIG. 2. Second oldest larva "B" of *P. filosa*; dorsal and ventral aspects of the head.

The third specimen (*C*) differed from "*B*" in the still more inflated "head" and in the replacement of the maxillipeds by a pair of tubercules, so that the ventro-anterior face of the "head" now presented five tubercules, one median and four paired. The rostrum of this stage pointed in a line with the axis of the "head" and its tip together with the tips of the anterior pair of lateral tubercules were visible from above. The dimensions of

"*C*" were; head 2.5 mm., thorax 132 mm., abdomen 12 mm. The "head" was 1.8 mm. broad at the widest; the horns spread 2 mm.

In the fourth member of my series "*D*" (Fig. 3) the chief change is the further progress of the shifting upward of the anterior face of the "head." The tips of the rostrum and anteriormost tubercules are visible from above as before. The antennæ have migrated to the upper surface of the "head" and the apices of the second pair are distant from the anterior border. These antennæ are adult in form. A few of the abdominal appendages consisted of a main ramus and two minute accessory rami, but the majority of this series of appendages had only a single accessory branch and the first five or six pairs were uniramous. The "head" of this specimen measured 3.5 mm., the body 130 mm., the abdomen, 16.5 mm.

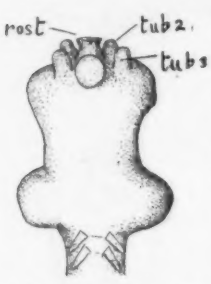

FIG. 3. Fourth larva "D" of *P. filosa*; ventral aspect of the head.

Of the two remaining specimens of the series; "E," the Woods Hole example, closely resembled "F" (Fig. 4), the last of the primary group. The "head" is now broad anteriorly, but does not as yet approach the rounded form characteristic of the "head" of the adult *P. filosa*. In "E" the antennæ were nearer the anterior margin of the "head" than was the case with "D," but were not visible from below. A trace of the cephalothoracic border is still present in this anterior dorsal region. The rostrum has disappeared and the venter of the anterior part of the "head" is covered with small tubercles, amid which the tubercules already introduced are lost. In specimen "E" the median one of the older series could just be identified. This specimen also exhibited faint traces of the median eye and the condition of its abdominal appendages was similar to that existing in "D," the six anterior pairs being unbranched, the remaining pairs having a longer outer and a shorter inner ramus. The rami are longer than the rami of "D's" appendages, however. All the abdominal appendages of "F" on the other hand had two

accessory branches and so further approached the complexly-parted appendages of the adult. The dimensions of "E" were; head 3 mm., thorax 150 mm., abdomen 9 mm. The head was 2 mm. wide and the horns spread 3.5 mm. The total length of "F" was 133 mm.

Although fundamentally similar, in several points the development shown by this series differs from the only previous description of undoubted *Penella* larvæ which I have been able to find, that recorded for *P. varians* by Steenstrup and Lütken ('61). This earlier account starts with a larva (Fig. 5) unquestionably much more immature than the youngest form in my series.

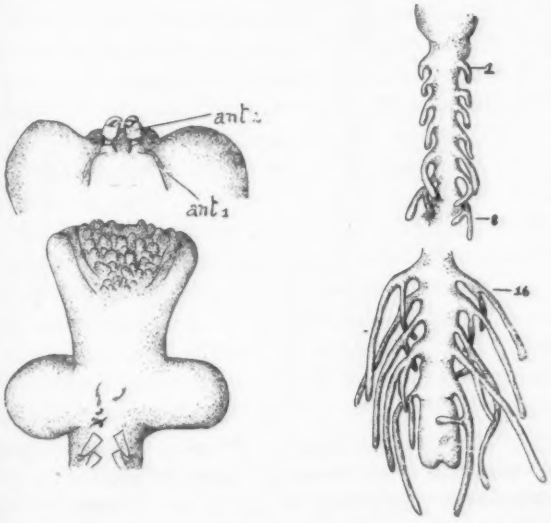

FIG. 4. Head of most mature larva of *P. filosa*; "F". Anterior and posterior extremities of the abdomen of larva "E".

This individual seems to have been about one centimeter in length, was filiform with a highly-convex cephalothorax, succeeded by three short thoracic segments, a long thoracic segment and a short, segmented (?) abdomen. This latter region lacked lateral appendages. The relative proportions of cephalothorax, thorax, and abdomen were — as shown in the figures — about 1′ 4′ 1′;

the posterior or "genital" thoracic segment being ten times as long as the three anterior rings together. The length of the thorax here shown proportionate to the cephalothorax and abdomen is in marked contrast to the excessively elongate thorax of the *P. filosa* larva "A," where the relative lengths of the three body regions are as 1′ 32′ 5′. However, the unlikeness should not be wholly referred to the difference in the ages of the two specimens, because in fully-mature specimens of *P. varians* the

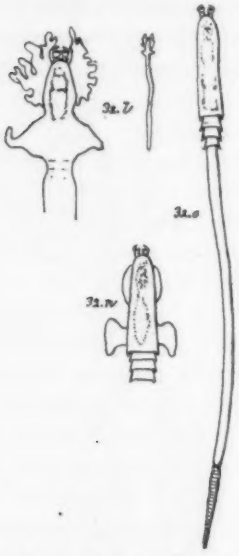

FIG. 5. Youngest, and second and fourth oldest larva of *P. varians*. (From Steenstrup and Lütken.)

FIG. 6. *Baculus elongatus*. (From Lubbock.)

thorax is proportionally much shorter than with *P. filosa*. The older larvæ of *P. varians* figured by the Danish observers show a gradual approach toward the proportions characteristic of the adult — 1′ 7′ 2′ as figured — although the abdomen remains in all disproportionately long. On the other hand, in the *P. filosa* series the thorax is about twice as long proportionally to the "head" and abdomen as in the adult; these regions only attaining their final dimensional relations later in the course of the development.

As with *P. filosa*, the horns of *P. varians* develop as a pair of outgrowths from the sides of the posterior part of the cephalothorax. The metamorphosis of the anterior part of this region of the body to form the "head" is also fundamentally similar in both species. In detail, the development shows noticeable differences. With *P. filosa* no division of the thorax into segments is traceable in my youngest larva, although this has no horns or "head." Later; the sides of the anterior cephalothorax become inflated, early destroying the convexity and distinct borders of the region. The mouthparts are gradually replaced by tubercules, the first of which to appear arise in a definite order. With *P. varians* (Fig. 5) the sides of the anterior region of the cephalothorax beneath the lateral borders of the shield early form two rounded outgrowths, while nearer the posterior end of this part of the body the horns protrude as two longer, pointed outgrowths. This occurs before the separate thoracic segments lose their identity and while the cephalothoracic shield is still distinct in all parts. In an older specimen of this species, the anterior or "head" outgrowths are shown to be broadened and have fimbriated margins. At this time the boundaries between the cephalothorax and thorax and those for the individual thoracic segments are no longer distinguishable while the borders of the cephalothoracic shield are still recognizable anteriorly and the mouthparts are intact. The later metamorphosis is not known.

The rostrum, mouthparts and antennæ of the *P. varians* larvæ seem to have been very like those described for my larvæ. The thoracic limbs are figured with the two anterior pairs bi-ramous, the two posterior pairs uni-ramous. No account is given of the changes which the cephalothoracic appendages undergo, nor of the development of the lateral appendages of the abdomen. It is clear that the latter were not present in any of the larvæ described and the implication is that their development is markedly retarded, although no definite statement is made with regard to this point. It is not improbable that in this species the adult shape of body and "head" may be attained before the abdominal appendages are developed. Such would appear to be the case with another species of *Penella* at least, *P. exocœti*, for Steenstrup and Lütken ('61) figure specimens which have a "head" of

almost adult form but lack abdominal appendages. The sexually mature *P. exocœti* evidently possesses abdominal appendages that are subdivided into several branches. The relative development of abdominal appendages compared with the "head" in the oldest specimen of the *P. filosa* series — "F" — suggests the possibility of a somewhat similar phenomenon here, to the extent that individuals may occur with the general appearance of an adult, but with less complexly cleft appendages. Such approaches in development to an adult bodily form before the abdominal structures have fully shaped themselves deserve notice, if only from the standpoint of the systematist. They may readily become not unimportant sources of confusion.

Moreover with *P. varians* the abdominal appendages are variable even in the adult animal, Steenstrup and Lütken recording them as bifid for several mature individuals (p. 411) although they are typically uniramous. With *P. filosa* the abdominal appendages of all the specimens of mature form that I have had opportunity to examine, were complexly subdivided into filamentous branches. The occasional presence of one or more simple or few-parted appendages at the anterior end of the series was the only variation found. This occurred in two out of seventy-nine specimens from *Orthagoriscus mola* in a collection loaned me by the Museum of Comparative Zoölogy. At least one of these was sexually mature and bearing egg-sacs and the same was true for a single specimen out of fifteen examples from the swordfish, which individual had three of the anteriormost abdominal appendages tri-ramous only. The number of the abdominal appendages is fairly constant in *Penella filosa;* varying from 21 to 30 pairs. Twenty-two or twenty-five pairs are the commonest. Of my larvæ: "A" had 22 pairs, "B" 21, "C" 23, "D" 21, "E" 20 and "F" 22.

On comparing the youngest *Penella* larva known, the immature *P. varians* figured by Steenstrup and Lütken, with that stage of the ontogeny of *Lernæa branchialis* at which fertilization and attachment to the final host takes place — the "begattungsstadium" of Claus' ('68) description — or to the corresponding phases of other lernæoid copepoda, the resemblance in general structure is striking. And it appears probable, therefore, that

the larva of *Penella* at the time when it attaches to the host has a form not unlike this "mating-stage" of the *Lernæa*. That is, it will closely resemble the youngest stage of the *Penella varians* series with the exception that the posterior or genital segment of the thorax will not be as disproportionately long in comparison to the rest of the body. Very probably this segment will not be sharply distinguished from the abdomen, as in the case in *Lernæa* of this age. A number of years ago Wierzejski ('77) described certain larval Copepods from the gills of *Loligo*, *Sepia* and *Eledone* at Triest and suggested that they might be the mating-stage and younger larvæ of *Penella varians*. His opinion was based on the characters exhibited by the appendages. As figured, however, these structures do not appear to be at all conclusive with regard to this hypothesis and in general form the larvæ certainly do not at all correspond to what would be inferred for the mating-stage of any *Penella*. Two minute immature Copepods have been recorded which seem to fill the requirements better. These were described under the names *Baculus elongatus* (Lubbock, '60) and *Hessella cylindrica* (Brady, '83). The former was collected in the Gulf of Guinea, the latter off Zamboanga in the Sulu Archipelago. Although not wholly alike in all structural details, they agree in possession of a filiform shape, cylindrical cephalothorax with projecting second antennæ, short segmented thorax, with four pairs of limbs, and an elongate hind-body devoid of lateral appendages and evidently representing a fusion of the posterior part of the thorax with the abdomen. Lütken in 1892 strongly urged that *Baculus elongatus* be regarded as a larva of *Penella*. He points out that *Baculus elongatus* is a pelagic form, in harmony with the fact that the genus *Penella* infests "fishes or whales with a more or less marked pelagic habit (*Xiphias*, *Coryphæna*, *Exocœtus*, *Diodon*, *Mola*, *Pterophryne*, *Hyperoödon*, *Balænoptera*)." The filiform shape of these larvæ unquestionably suggests *Penella* and the argument from habitat is significant. But, nevertheless, it must be admitted that on a basis of present knowledge of the forms a definite determination is not possible. We can only say that *Baculus* or *Hessella* are almost certainly the young of lernænoid copepods, possibly of *Penellas*. However this may be,

these larvæ are helpful in constructing a picture of the hypothetical mating-stage phase of *Penella's* metamorphosis.

Attached to the host while possessing such a *Baculus*-like form, probably at first by the antennæ, then later burrowing into the tissues, the retrogressive metamorphosis begins. An elongation of the posterior thoracic segment brings about a condition in which the young *Penella* will parallel the earliest described stage of *P. varians*. Then the adult form is gradually developed. The anterior part of the cephalothorax moulds itself into the "head," the horns protrude from the posterior part of this region, the thoracic segments coalesce, the anterior ones remaining undeveloped, the posterior elongating to form the major part of the body, while the abdomen develops the characteristic lateral appendages.

As regards the metamorphosis prior to the attainment of the mating-stage form, we only know that the young *Penellas* almost unquestionably hatch as nauplii. The analogies presented by the development of *Lernæa* and allied copepods (Scott, '01, Claus, '68) suggests the following cycle in the period between the nauplius and the completed, fertilized, mating-stage larva. The nauplius probably metamorphoses to a cyclops-like form, the so-called "*Chalimus*" stage, and in this phase the young attach themselves to some host. Typically among parasitic copepods, the attachment in this chalimus stage in made by a frontal filament. While thus fixed on the host, the mating stage is reached by successive moults. At this point in the ontogeny of *Lernæa* the male and females copulate and the latter detach themselves and for a short period return to a pelagic life. Then they settle down upon the final host, which happens to be a different kind of fish from the one they infested during the earlier part of their life. Other parasitic copepods pass through a similar life cycle on a single species of animal as host. Of course we have no clue to which of these groups *Penella* belongs, but its Lernænoid affinities suggest a metamorphosis with the double host. Nevertheless, this possibility should in no way discourage the most careful scrutiny of freshly captured fishes that are infested with *Penellas* or are species which serve as hosts for this genus, if opportunities present themselves. The metamorphosis

must run a course similar to that in *Lernæa*, and if only a newly-attached mating-stage larva could be identified and described, it would go far toward the unravelling of the life-history of these highly-modified copepods.

Similarly, the discovery of the males for this genus is much to be desired. Steenstrup and Lütken ('61) record finding near the egg-sacs of a *Penella exocœti* two almost microscopic animals which reminded them of the "pigmy males of other lernæan forms." They figure one of these with the suggestion that it may be the vainly sought male. The specimens could not be removed for study. It is very doubtful whether this is a male of *Penella*. It would seem that the male of this genus ought rather to resemble the male of *Lernæa* and its form will be cyclopoid much like that of the chalimus stage of the females. As with *Lernæa* also, it is unlikely that the males remain attached to the females after fertilization has taken place, but continue on the host which has served them for their earlier development, while the females become free swimming after fertilization and settle down on another animal of the same or another species from the first host.

CLARK UNIVERSITY.

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THE REARING OF SERPULID LARVÆ WITH NOTES ON THE BEHAVIOR OF THE YOUNG ANIMALS.[1]

CHARLES ZELENY.

The Method of Rearing.

The complete success of an attempt to rear serpulid larvæ[2] has prompted the present description of the method, in view of the fact that other workers have been unsuccessful in the same field.[3] It is hoped that the method may be found valuable not only for serpulid larvæ, but also for the great majority of forms which do not allow a direct current of water to be passed in and out of the dish containing them.

The eggs after fertilization were placed in large glass beakers and the water was changed several times during the first day. They were then allowed to develop into free swimming trochophores which collected in great numbers at the surface of the water during the second day. A few of these were removed with a pipette and placed in a "battery jar" containing fresh sea water obtained in the open harbor at rising tide on a bright afternoon. Not more than a few hundred larvæ were put into any one battery jar. The jars were covered with glass plates to keep out the dust, and were placed in such a position that the rising sun shone on them for about an hour just after sunrise each morning. Immediately after the sun bath they were cooled off in buckets of cold spring water and were then placed for the rest of the day in shallow basins of the same cold water. In this way the proper conditions for the development of the microscopic algæ and other organisms which serve as the food of the larvæ

[1] Contributions from the Zoölogical Laboratory of Indiana University, No. 65.

[2] *Hydroides dianthus.*

[3] The work described was done at the Cold Spring Harbor Biological Station during the summer of 1902, while the writer was holding the John D. Jones Scholarship at that place. My best thanks are due to Professor C. B. Davenport, the director of the station, for his kindness in supplying me with every possible facility while at the laboratory. Some of the notes refer to work done at the Naples Zoölogical Station during the winter of 1902–3.

were furnished without at the same time heating the water to such an extent as to kill the larvæ themselves.

Under the above treatment the larvæ flourished and successfully went through the transformation from the free-swimming to the sedentary condition. They settled down on the sides of the vessels in great numbers about fourteen days after fertilization and observations could readily be made upon them with a horizontal microscope. The examination of the larvæ can undoubtedly be facilitated by the suspension of glass slides in the water at the places where the free-swimming larvæ are about to attach themselves. The slides with the attached larvæ can then be transferred to shallow dishes of sea water and examined with the microscope in its ordinary upright position.

A study of the development of the opercula in the serpulids constituted my main object in rearing the larvæ and a description of this feature is incorporated in a paper now in press.[1] A few incidental observations on the behavior of the young animals are given in the following notes :

Behavior of the Young Serpulids.[2]

The Free Swimming Larvæ. — As soon as the pre-oral band of cilia is well developed the young larvæ swim toward the surface of the water and collect there in great numbers, especially at the edges near the glass sides of the jar. They are always more crowded in certain regions than in others, but the exact relation of this crowding to a phototactic response was not made out because of the complex relations due to refraction and reflection of the light within the jar. The greatest crowding was usually on the side of the jar facing the window and on the side directly away from the window. At the latter place the collection of larvæ may have been due to a secondary reflection of the light within the jar.[3]

[1] "Compensatory Regulation." To appear in the *Journal of Experimental Zoölogy*, Vol. II., No. 1.

[2] *Hydroides dianthus* (at Cold Spring Harbor), unless otherwise noted.

[3] A. Giard ('76) in his "Note sur l'embryogénie de la Salmacina Dysteri Huxl." (*Compt. Rend.*, Tome 82, 1876, pp. 233–235, 285–288) says that the free-swimming larvæ of *Salmacina* collect on the sides of the aquarium facing the light while they settle down and form tubes on the side away from the light.

Attachment of the Larvæ. — The period at which attachment of the larvæ should take place is a critical one and death is the usual result under ordinary laboratory conditions. In my experiments at Cold Spring Harbor, however, there were hundreds of survivors in each jar. On account of the frequent change of position of the jars, as well as the complex refractions and reflections within them, I was not able to find out whether the place of attachment of the larvæ has any definite relation to the source of the light. The young Serpulids were found to be fairly uniformly distributed, though there were groupings at several different places. One of these consisted of a large number collected on the glass just below the surface film of the water. These formed a band surrounding the jar, but it is interesting to note that they did not grow as rapidly as those lower down, although fresh sea water was added in each case to raise the level slightly and make up for evaporation. Going down from this band-like zone of greatest frequency the number of individuals decreased until the bottom of the vessel was reached, where again there was a considerable number, especially in the corner between the bottom and the sides of the jar.

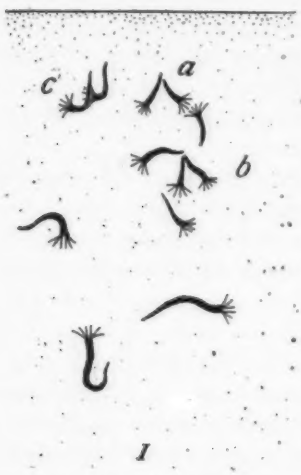

FIG. 1. (× 4.) A group of the young of *Hydroides dianthus* as attached to the side of a battery jar. The level of the water surface is shown. *a* and *b* are groups showing radial divergence of the tubes. *c* shows two tubes which have evidently responded to a lateral stimulus. No definite relation to gravity is shown.

Tube Formation. — The tube when first formed is a very narrow, almost transparent ring of calcareous matter, the body of the short larva extending out of it at both ends. This ring is secreted by the region just back of the free anterior end of the thoracic membrane and as its formation goes on the animal can be seen to extend its thoracic membrane over the anterior edge of the tube in order apparently to smooth the edges and get the

material in shape to fit the body. At such a time the body may project a considerable distance from the anterior end of the tube. The tube is deposited quite rapidly. In the case shown in Fig. 2 the amount of growth in the course of twenty hours is given. This is equal to .29 mm. or .35 mm. per day.

I tried to discover some regularity in the arrangement of the axes of the tubes with respect to gravity, light and food conditions but was able to find no general rule in the matter although some groups seem to be arranged with respect either to maximum food-obtaining ability or with respect to a lateral stimulus of unknown character. Fig. 1 which is reproduced from my notebook gives a small section of a side of one of the jars. Evidently there is no general rule of arrangement though radial divergence of the kind shown at *a* and *b* (Fig. 1) may be explained on a utilitarian basis as a spreading out from a central point in order to obtain more feeding room. The arrangement at *c* however does not come under this head but must be considered as a very definite response to a lateral stimulus. It is hard to conjecture what this stimulus may have been.

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FIG. 2. (× 38.) Open end of a tube of *Hydroides uncinata*. The unshaded portion represents the amount of addition to the tube in the course of twenty hours.

General Activities of the Young at a Later Stage. — The following observations were made on young individuals during the few days preceding and the few days following the formation of the operculum. Specimens of *Hydroides uncinata* and *H. pectinata* observed at the Naples Zoölogical Station during the winter of 1902–1903 as well as the *H. dianthus* of Cold Spring Harbor were used.

The two eye spots are very prominent at this stage and a sudden shadow such as is produced by the passing of a hand between the animals and the source of light causes the serpulids to contract and withdraw with a jerk into their tubes. The branchiæ are then completely hidden inside of the tube and before the development of the operculum their ends form a barricade a short distance within the opening. After a short period of inaction if there is no new disturbance the fine thread-like

ends of the branchiæ begin to appear and wave back and forth around the mouth of the tube as if feeling for signs of danger. In this position the animal usually remains for several minutes often with nothing more than the ends of the two longest branchiæ projecting from the tube. After satisfying itself in this manner the animal pushes itself forward and expands the whole branchial crown with comparative rapidity. After the development of the functional operculum this organ is pushed to one side and the branchial ends are slightly projected around its margin before the thrusting out of the whole branchial circlet takes place (see Fig. 3). The young animals, as well as the adults, are extremely sensitive to the slightest mechanical jar, a very small shock being sufficient to keep them within their tubes for a considerable time. At Naples I was often greatly annoyed while attempting to make drawings of the animals in an extended state to see them jerk back into their tubes at the sound of a band starting to play in the adjacent park. Under such conditions they "sulked" in their tubes until the musical selection was finished.

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FIG. 3. (× 38.) *Hydroides pectinata*. The young serpulid has thrust out the ends of its branchiæ preparatory to the expansion of the whole branchial circlet.

INDIANA UNIVERSITY,
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